



Automated Protein Buffer Exchange Enables High-Throughput Thermal Shift Assay and Streamlined Liquid Formulation Development

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INTRODUCTION

- Developing stable protein formulations is essential for successful biologic products, yet traditional formulation screening often requires weeks to months of iterative experimentation, significantly increasing development timelines and costs.
- Integrating automated liquid handlers (ALH) with Affinity and SizeX IMCStips® enables rapid protein purification and buffer exchange into 96 unique formulations directly from cells.
- Protein stability can then be evaluated using differential scanning fluorimetry (DSF) with SYPRO™ Orange dye on a real time-PCR platform, enabling a high-throughput method to screen proteins based off melt temperatures (T_m).
- This approach provides a rapid, scalable method to assess protein thermal stability through T_m values and can be incorporated into Design of Experiments (DoE) workflows to identify and optimize formulation parameters, accelerating the path towards robust and stable protein products.

METHODS

Table 1. Protein abbreviations.

Protein Name	Abbreviation
<i>Neisseria meningitidis</i> CMP sialic acid synthetase	NS
<i>Bifidobacterium longum</i> spp. <i>infantis</i> β -1,3-D-galactosyl-D-hexosamine phosphorylase	BP
<i>Brachyspira pilosicoli</i> β -glucuronidase	BE
<i>Bifidobacterium luumgum</i> UDP-sugar synthase	BL

Table 2. Buffers used for high throughput screening.

Buffer	pH
Sodium citrate (Na Citrate)	6.0
HEPES	6.5, 7.0, 7.5
Tris-HCl	8.0, 8.5
Glycylglycine (Gly-Gly)	8.5, 9.0
Glycine	9.0

Table 3. Chemicals used for formulation screenings.

Chemical	Concentration
NaCl	0, 125, or 250 mM
Sucrose	5% (w/v)
Sorbitol	5% (w/v)
Glycerol	5% (w/v)
L-Arg	20 mM
L-His	20 mM
MgCl ₂	10 mM

- Frozen cells were thawed and lysed using B-PER Complete.
- Lysate was centrifuged and supernatant was diluted with buffered solution.
- Proteins were purified from cell lysate using Ni-IMAC IMCStips on automated liquid handlers (ALH).
- Purified proteins were buffer-exchanged into 96 formulations using SizeX IMCStips.

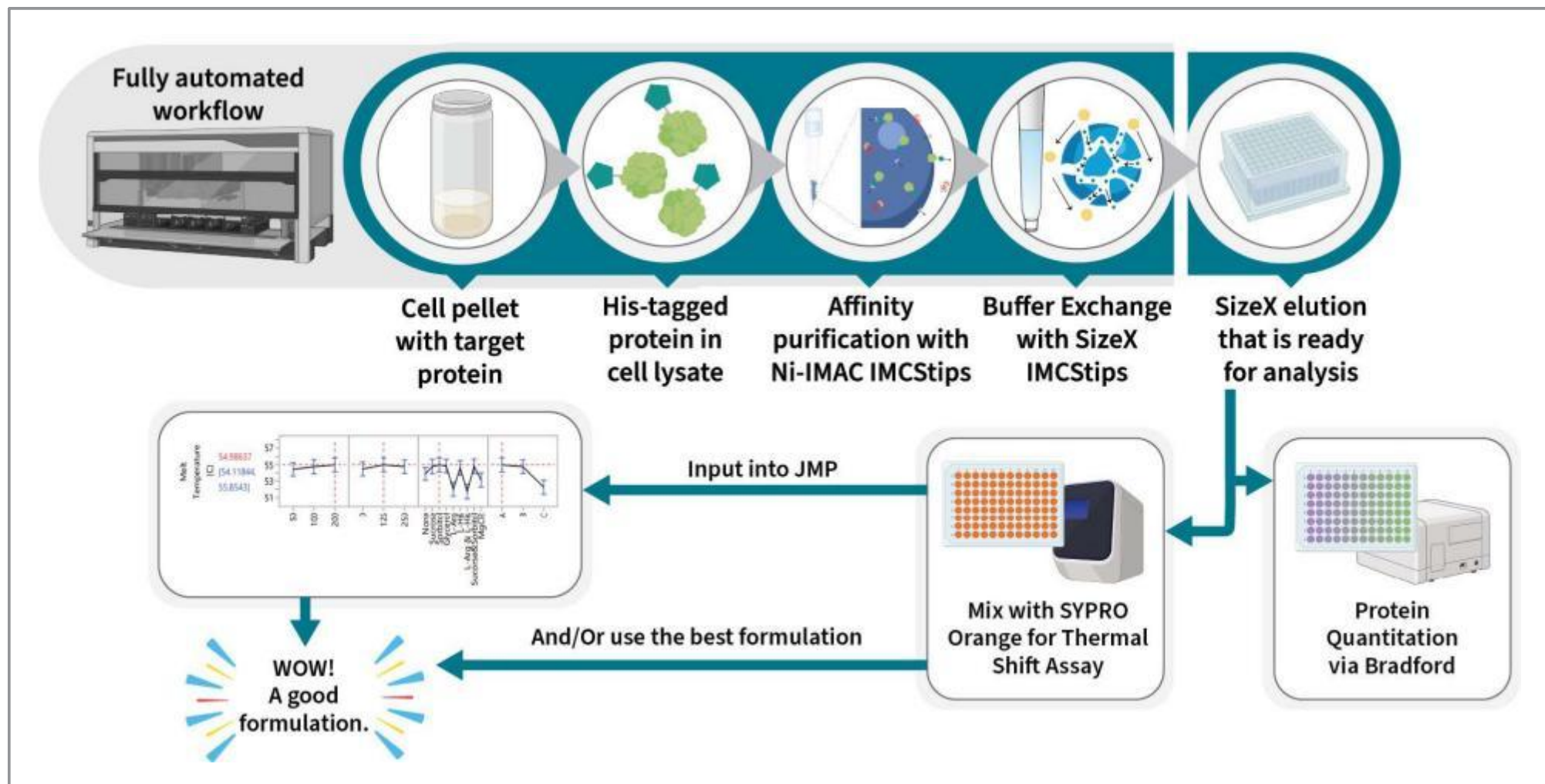


Figure 1: Workflow scheme.

- Formulated proteins were quantified by Bradford assay with BSA as a standard.

- SYPRO orange was mixed on ALH deck.

- Thermal stability was measured by differential scanning fluorimetry (DSF) with SYPRO Orange dye on the StepOnePlus RT-PCR and compared to differential scanning calorimetry (DSC) with TA DSC2500.

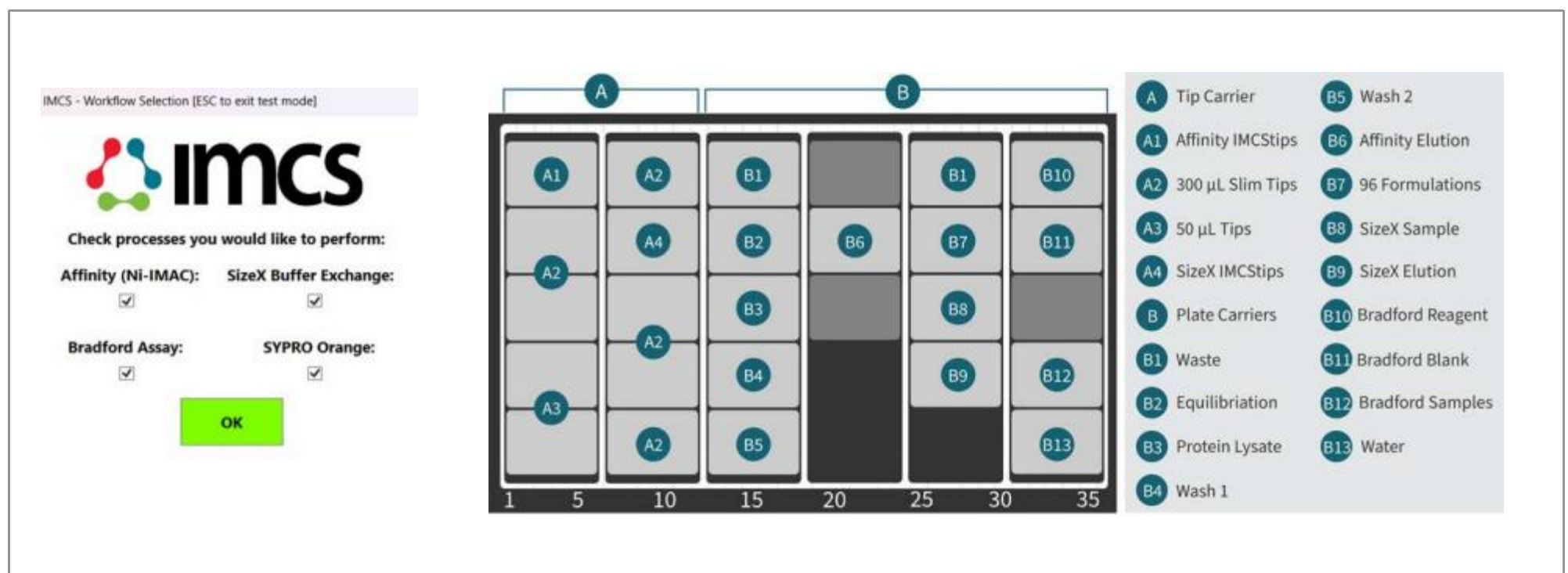


Figure 2: Deck layout on automated liquid handler for protein purification followed by buffer exchange into 96 different formulations. Formulated proteins are then aliquoted for protein quantification using Bradford assay and for SYPRO Orange thermal shift assay.

- Upload of initial screening results to JMP Design of Experiments (DoE) identified optimized formulations for subsequent experiments.
- Enzyme activities were measured over time at multiple temperatures for proteins stored in three different formulations: two highest T_m formulations and one lowest T_m formulation as a negative control.

RESULTS

Initial Screening of Formulations

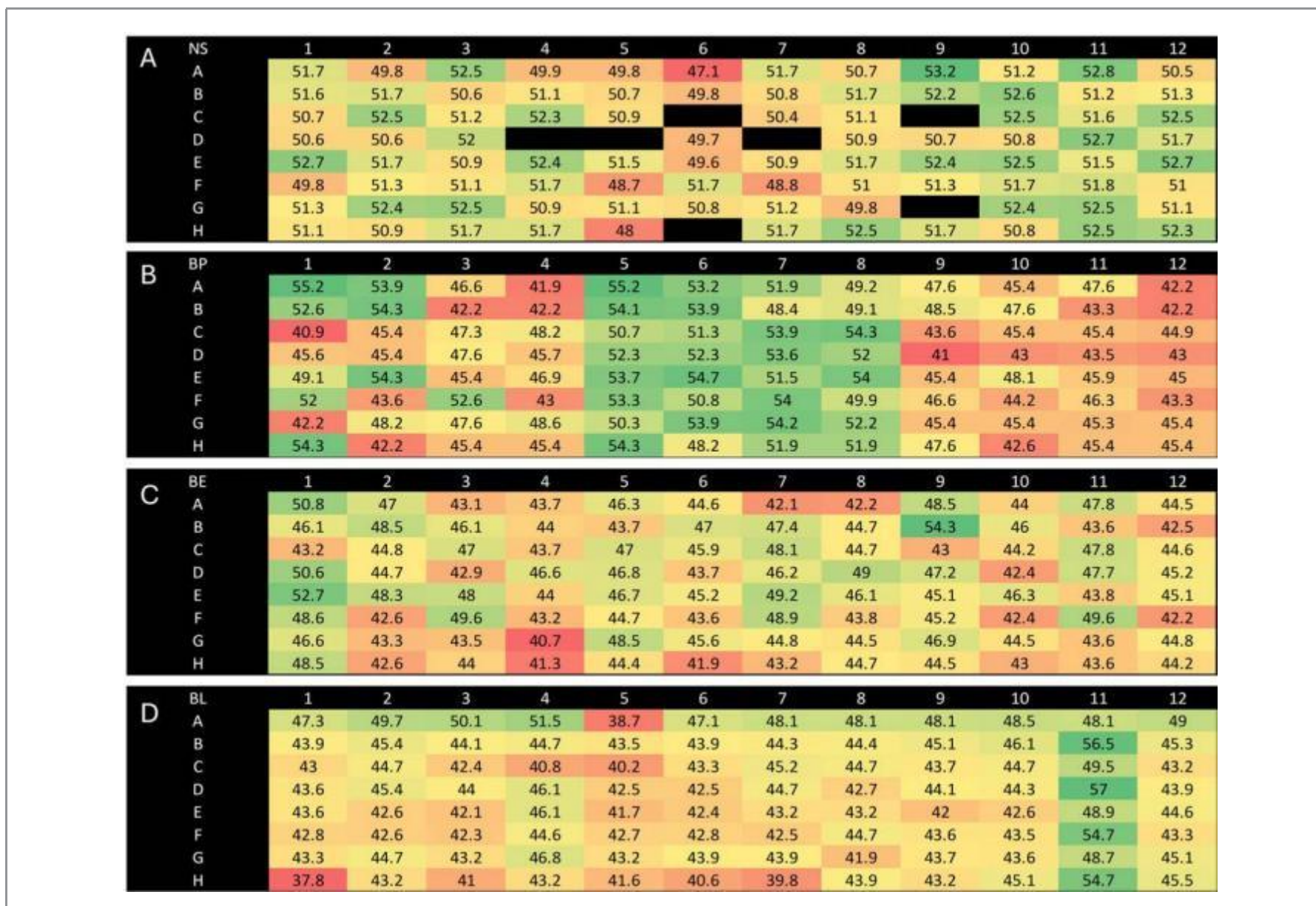


Figure 3. Initial screening of formulations. Melting temperatures (T_m) for (A) NS, (B) BP, (C) BE, and (D) BL in 96 different formulations. Color scale represents temperature magnitude, with green indicating higher T_m and red indicating lower T_m .

Formulation predictions on JMP with DoE

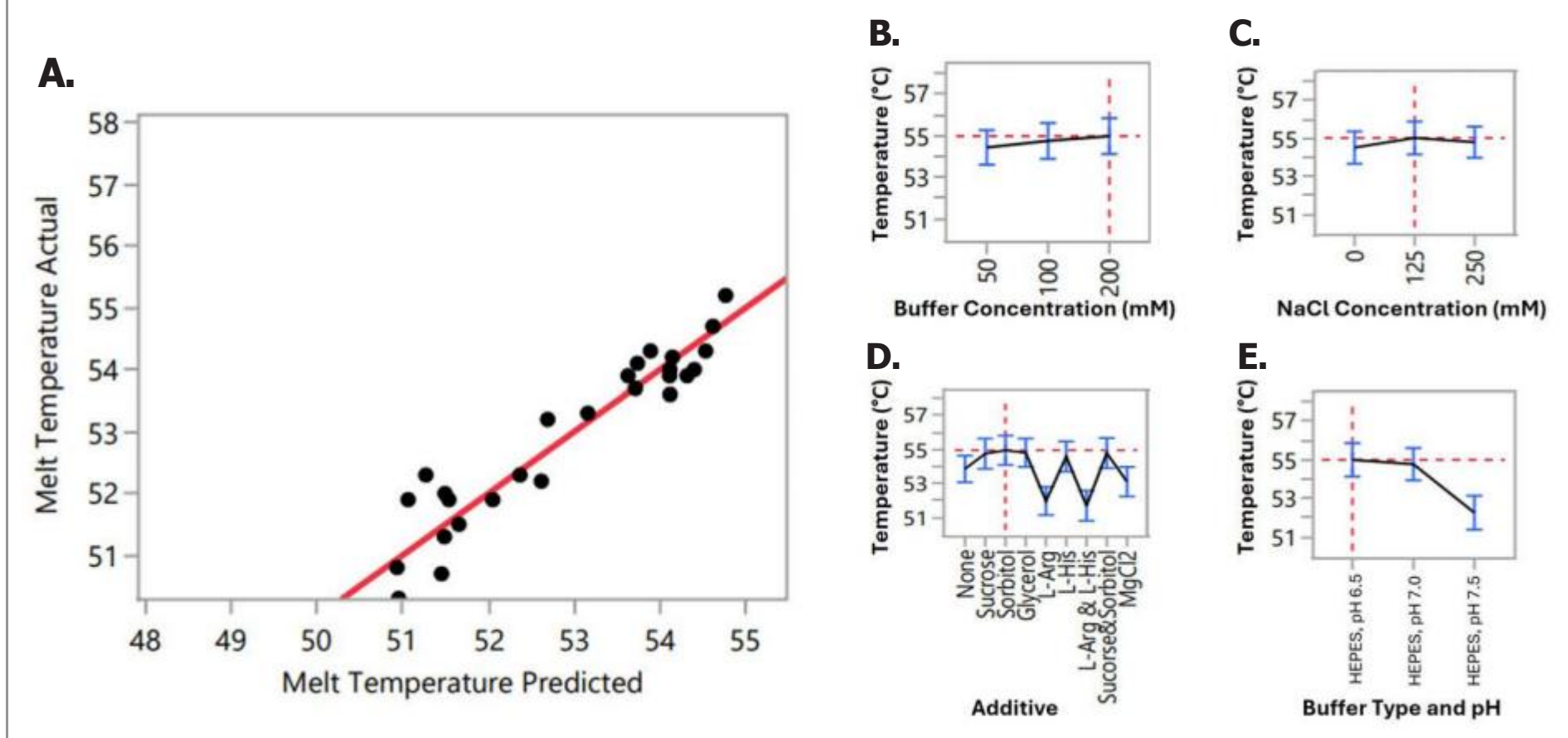


Figure 4. (A) Correlation between predicted and measured melt temperatures (T_m) from the DoE model. Prediction profiler predicting the impact of (B) buffer concentration, (C) NaCl concentration, (D) additives, and (E) salt type and pH on T_m values.

Formulations selected after initial screening

Table 4. Formulations used for each enzymeduring stability testing.

NS			BP		
NS-1	50 mM Gly-Gly, pH 8.5, 250 mM NaCl, 5% glycerol	T _m : 54.1°C	BP-1	200 mM HEPES, pH 6.5, 125 mM NaCl, 5% sorbitol	T _m : 55.0°C
NS-2	200 mM Gly-Gly, pH 8.5, 250 mM NaCl, 5% sorbitol	T _m : 53.2°C	BP-2	200 mM Na. Citrate, pH 6.0, 250 mM NaCl, 5% sorbitol	T _m : 55.2°C
NS-3	100 mM HEPES, pH 6.5, 250 mM NaCl, 10 mM MgCl ₂	T _m : 47.1°C	BP-3	50 mM Na. Citrate, pH 6.0, 125 mM NaCl, 20 mM L-Arg	T _m : 40.9°C

Stability of NS

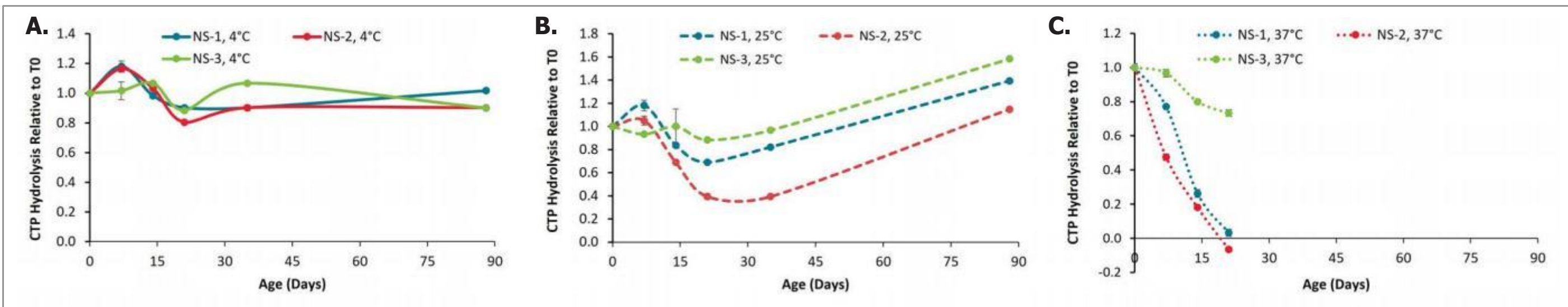


Figure 5. Relative activity of NS in three formulations stored at 40C (A), 220C (B), and 370C (C) over time in days.

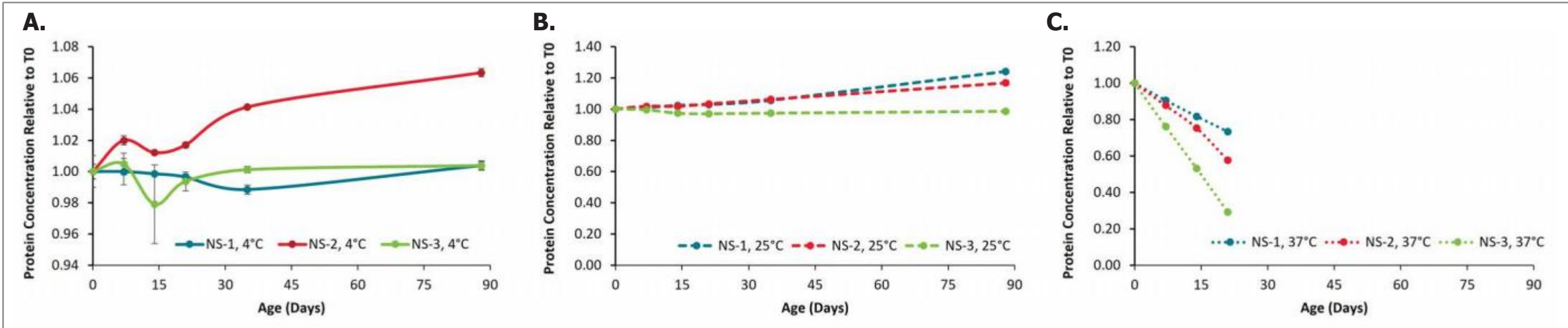


Figure 6. NS protein concentrations in three formulations stored at 40C (A), 220C (B), and 370C (C) measured across time in days.

Stability of BP

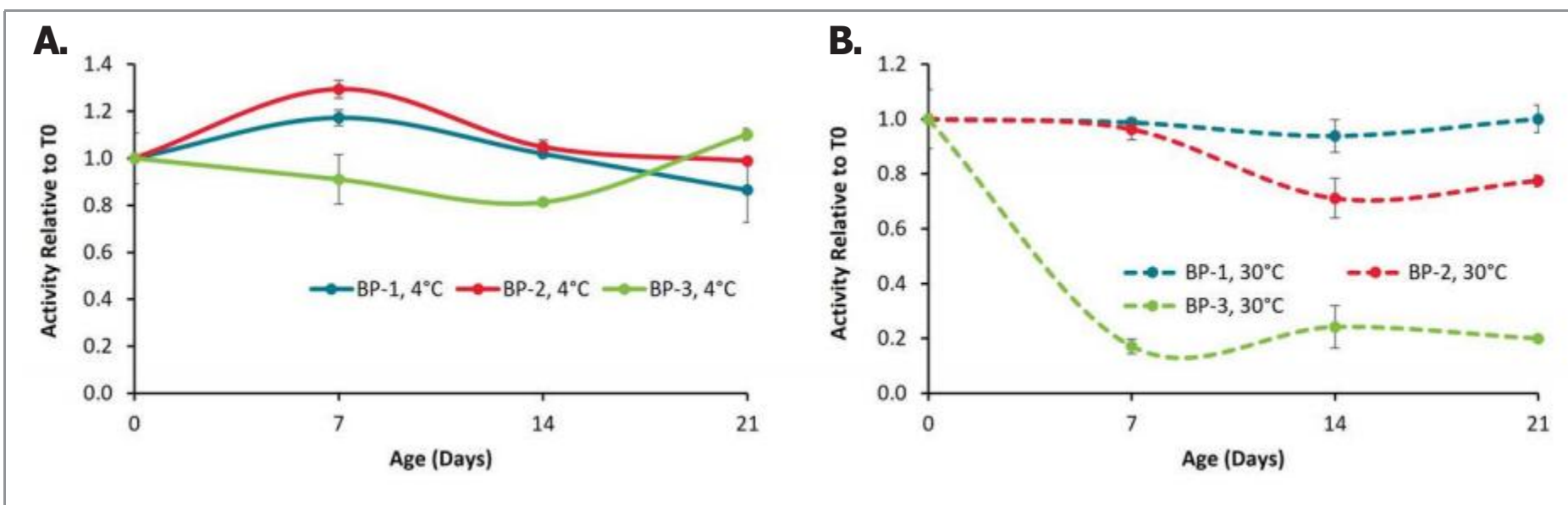


Figure 7. Relative activity of BP in three formulations stored at 40C (A) and 300C (B) over time in days.

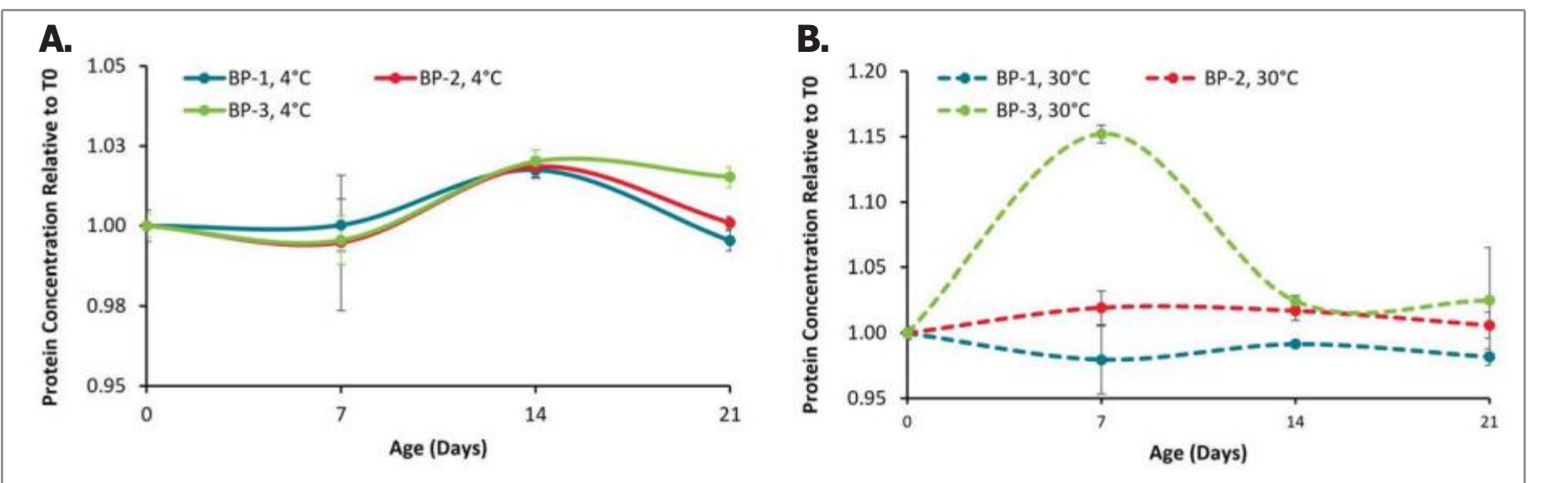


Figure 8. BP protein concentrations in three formulations stored at 40C (A) and 300C (B) over time in days.

Melt Temperature Variation between DSF and DSC

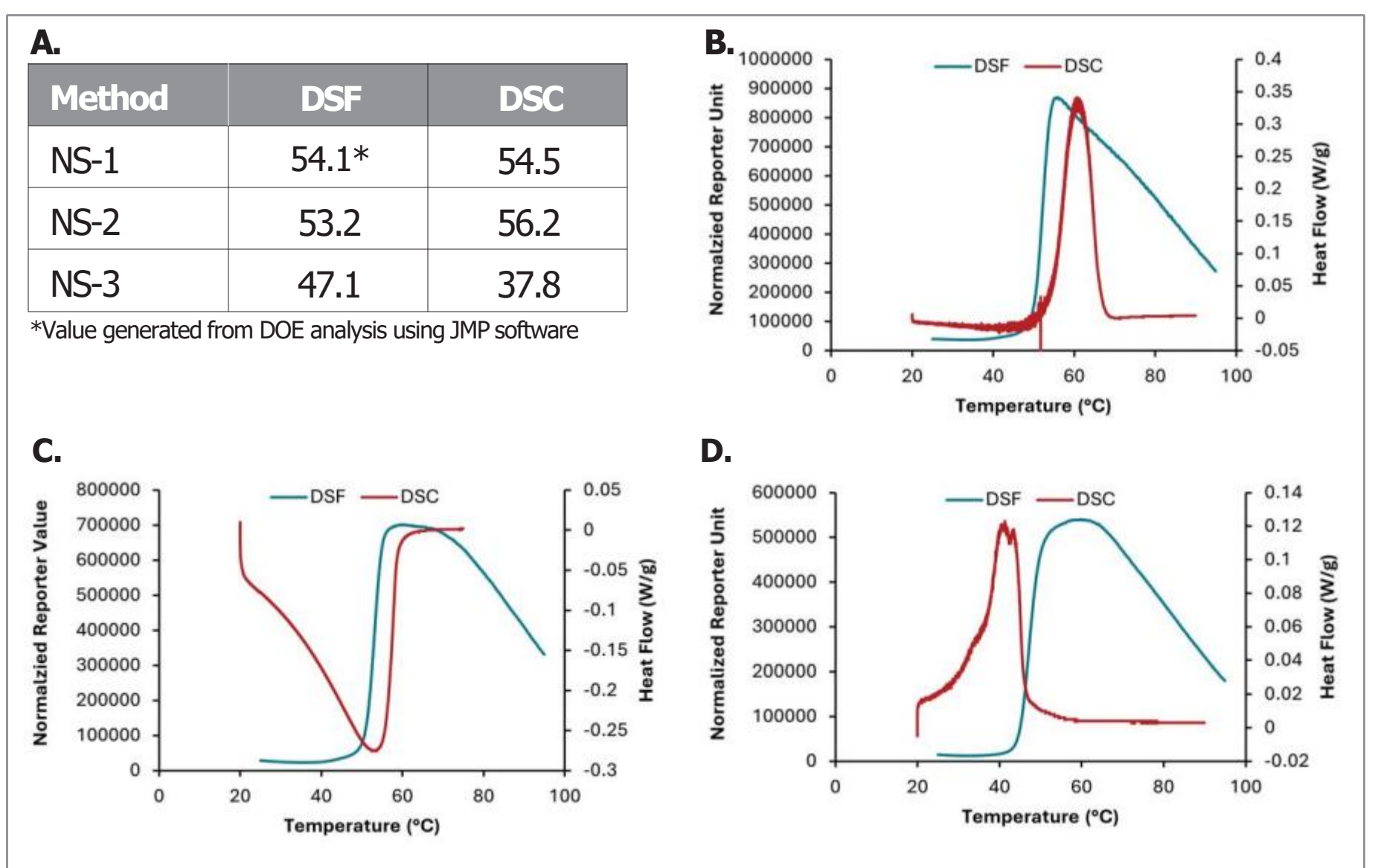


Figure 9. Melt curves for NS from DSF with SYPRO Orange dye and from DSC. (A) Melt temperatures calculated from DSF and DSC for three formulations. (B) DSF and DSC melt curves for NS in NS-1, a DoE optimized formulation. (C) DSF and DSC melt curves for NS in NS-2, the high performing formulation during DSF screening. (D) DSF and DSC melt curves for NS in NS-3, the worst performing formulation during DSF screening.

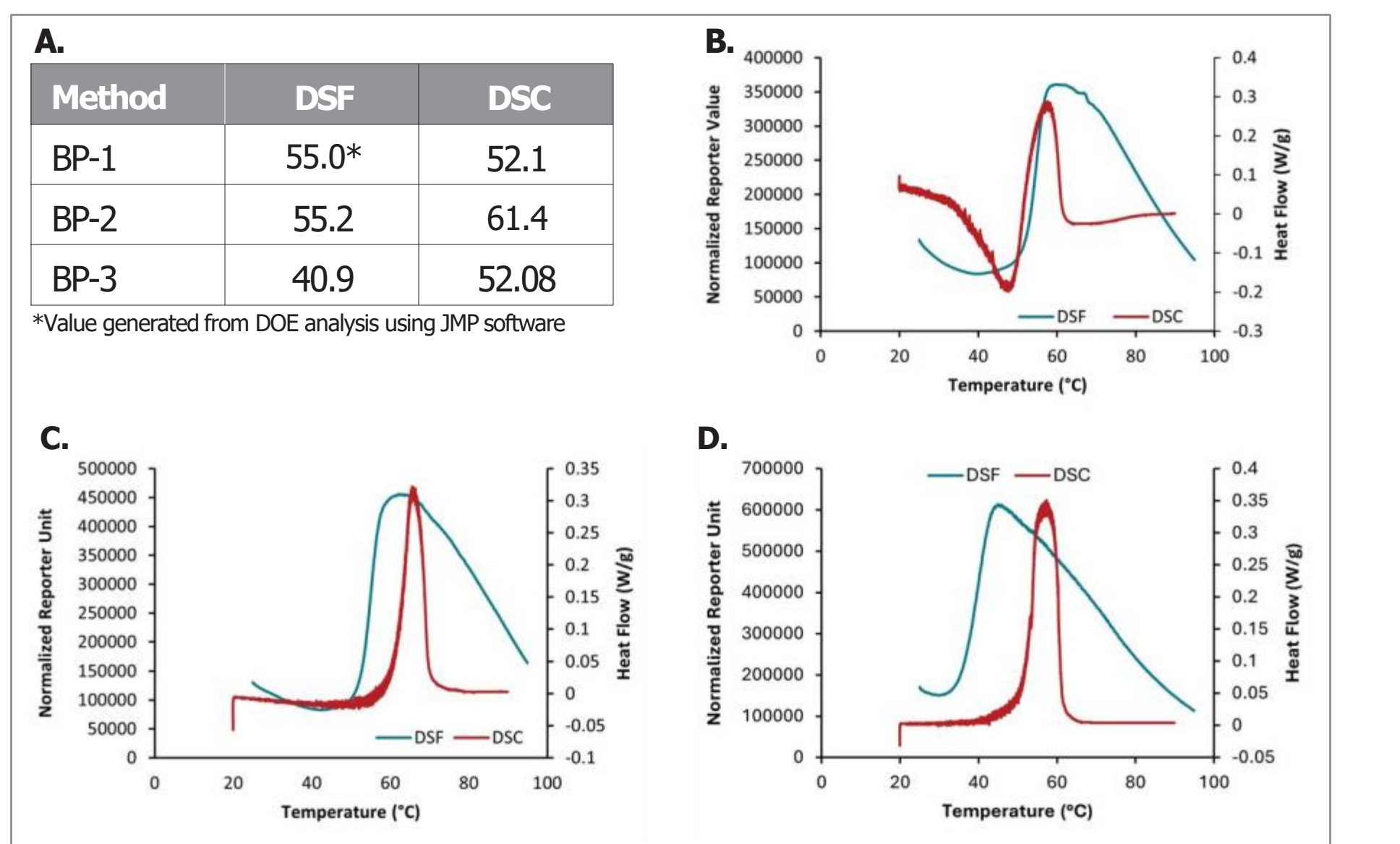


Figure 10. Melt curves for BP from DSF with SYPRO Orange dye and from DSC. (A) Melt temperatures calculated from DSF and DSC for three formulations. (B) DSF and DSC melt curves for BP in BP-1, a DoE optimized formulation. (C) DSF and DSC melt curves for BP in BP-2, the high performing formulation during DSF screening. (D) DSF and DSC melt curves for BP in BP-3, the worst performing formulation during DSF screening.

CONCLUSION

- IMCStips enabled purification and buffer exchange across multiple protein samples while significantly reducing costs relative to traditional workflows.
- By using automated liquid handling (ALH), 96 samples can be buffer exchanged at the same time into different formulations versus single buffer exchange by conventional Fast Protein Liquid Chromatography (FPLC).
- Formulations providing most stability quickly identified through Differential Scanning Fluorimetry (DSF) screening with SYPRO orange.
- This workflow is readily scalable to support higher-throughput screening and the generation of structured datasets.
- Automating workflows and datasets can enable Machine Learning-driven approaches for identifying novel protein formulations and accelerating biologics development.

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